CAMPYLOBACTER
IN CHICKEN LIVER

NSW GOVERNMENT Food Authority

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Contents

Introduction

A key result area for the NSW Food Authority (The Food Authority) is that safe food is produced and sold in NSW. The Food Authority has a strategy to identify and investigate contributors to foodborne illness with the aim of reducing foodborne illness in the community.

Transmission and Symptoms

Campylobacteriosis is a zoonosis; it spreads from infected animals to humans, and occasionally from person to person. An animal or a host becomes infected and extremely large numbers of the bacteria are excreted in the host's faeces. The bacteria can then spread to humans via water, food or direct contact. The infective dose for *Campylobacter* can be as low as 500-600 cells (Wallace, 2003).

The symptoms of campylobacteriosis generally last from two to 10 days and diarrhoea (sometimes bloody), vomiting, and cramping are usually self limiting in people who are otherwise healthy. However, a small percentage (about 0.15%) of patients develop complications that may be severe. These include bacteria entering the blood stream and infection of various organ systems, such as meningitis, hepatitis, cholecystitis, and pancreatitis. *Campylobacter* infection is also associated with long-term sequelae, including Guillain-Barre syndrome, reactive arthritis and irritable bowel syndrome (Wagenaar, French, & Havelaar, 2013).

Incidence

The Australian National Notifiable Disease Surveillance System table reported that on average, 19,000 cases of campylobacteriosis were notified per year over the last decade (http://www9.health.gov.au/cda/source/cda-index.cfm). In 2017, there were over 28,000 cases notified [Note that in NSW, campylobacteriosis has only been notifiable since April 2017].

Campylobacter is one of the most common bacterial causes of human gastroenteritis in the world (ESFA, 2014; FAO & WHO, 2009). Often it causes sporadic cases of illness rather than large scale outbreaks (Taylor et al., 2013). Because of this, it is widely accepted that campylobacteriosis is under-reported and studies have been conducted to estimate the true incidence.

In Australia, Hall, Yohannes, Raupach, Becker, & Kirk (2008) estimated that 225,000 cases of foodborne campylobacteriosis occurred in Australia per year from 2000 to 2004. Kirk, Ford, Brown, & Hall (2014) revised this estimate to 139,000 cases in 2000 and 179,000 cases in 2010. Stafford et al. (2008) estimated that 50,500 cases of *Campylobacter* each year in Australia could be attributed to the consumption of chicken (both undercooked and apparently well cooked, based on colour).

In the European Union, 198,252 cases were reported in 2009, but the true incidence was estimated at 9.2 million cases (Havelaar, Ivarsson, Löfdahl, & Nauta, 2013). In the United States, Wagenaar et al. (2013) suggested an annual incidence of 1.3 million cases. In many countries, the organism is isolated three to five times more frequently than other gastroenteritis causing bacteria; for example, in Switzerland, *Campylobacter* is isolated five times more than *Salmonella* (Baumgartner, Felleisen, & Gut, 2012).

When taken together, the various illnesses triggered by *Campylobacter* result in a great deal of lost time, considerable disability and some deaths (Wagenaar et al., 2013). The European Food Safety Authority (2014) estimates that the cost of lost productivity due to campylobacteriosis in Europe is around €2.4 billion a year. For example, the burden of campylobacteriosis in the Netherlands in 2009 was the second highest of the foodborne pathogens (Havelaar et al.,

2012). In the United States, campylobacteriosis is estimated to cost US$1.56 billion annually (Scarff, 2012) and in the UK in 2008-2009 the economic cost of campylobacteriosis was estimated at £50 million (Tam & O'Brien, 2016).

Closer to home, in New Zealand the food-attributable estimated cost of illness for campylobacteriosis and its sequelae was NZ$74 million (Lake, Cressey, Campbell, & Oakley, 2010). This estimate applies to illness rates prior to New Zealand's *Campylobacter* interventions.

Campylobacter and poultry

Despite the variety of exposures to *Campylobacter*, there is a broad scientific agreement that poultry meat is a major transmission vehicle, and most probably the leading vehicle, in most countries for campylobacteriosis.

The combination of *Campylobacter* and poultry was the highest-ranking food/pathogen combination in the United States with an estimated annual burden of 608,231 illnesses, 6091 hospitalizations, 55 deaths and cost of illness at US$1.26 billion (Batz, Hoffman, & Morris, 2012).

Globally there is a focus on reducing the prevalence of *Campylobacter* in raw poultry at farm and abattoir with interventions such as increasing on farm biosecurity controls, minimising cross contamination from the intestinal tract during slaughter, air and water chilling, post slaughter rinses and storage conditions e.g. freezing livers to reduce *Campylobacter* prevalence in raw poultry (Harrison, Corry, Tchorzewska, Morris, & Hutchison, 2013; Lake et al., 2013; Northcutt, Berrana, Dickens, Fletcher, & Cox, 2003). NSW poultry processors in conjunction with the Food Authority have set key performance targets in the areas of process hygiene as specified by Food Standards Australia New Zealand Compendium of Microbiological Criteria for Food (FSANZ, 2018). This information is shared with the Food Authority with proactive action taken in response to trends.

Campylobacter can penetrate poultry livers which makes the core cooking temperature a critical food safety step (Moore & Madden, 1998; Whyte, Hudson, & Graham, 2006). Over the past few years there have been several *Campylobacter* outbreaks in Australia linked to pâté made from poultry livers (

Table 1). These outbreaks have been in the restaurant or catering settings (as opposed to commercially prepared pâté). Internationally there have also been many high-profile outbreaks of campylobacteriosis linked to pâté made from poultry liver (Appendix 1). Little, Gormley, Rawal, & Richardson (2010) stated that the number of outbreaks related to undercooked chicken liver pâté in England and Wales increased significantly from 2007 to 2010. In addition, because pâté is often used as a celebratory food, outbreaks from undercooked pâté seem to occur more often around holiday and festive times.

Contributing factors to outbreaks linked to undercooked chicken or duck pâté include:

- Cooking to a core temperature of 65°C but not holding it for the required length of time (Inns, Foster, & Gorton, 2010)
- Shallow frying livers to retain pink colour and only cooking to core temperature of 60°C (Edwards et al., 2014)
- Only lightly cooking the liver to retain pink colour (Abid et al, 2013; CDC, 2013; CDC, 2015; O'Leary, Harding, Fisher, & Cowden, 2008; Young et al., 2013)
- Using a bigger pot than normal but no adjustment on the cooking times to compensate for the larger pan (Wensley & Coole, 2013).

Table 1: Australian outbreaks of campylobacteriosis linked to chicken or duck pâté

Year	State	Vehicle	Cases (hospitalisation)	Setting	Reference
2016	NSW	Chicken liver pâté	3	Restaurant	Communicable Diseases Branch, 2017
2015	NSW	Chicken liver pâté	2 (1)	Restaurant	Communicable Diseases Branch, 2016
2015	NSW	Chicken liver pâté	2 (1)	Restaurant	Communicable Diseases Branch, 2016
2014	ACT	Chicken liver parfait	2 (1)	Restaurant	OzFoodNet, 2015c
2013	ACT	Chicken liver pâté	56	Commercial caterer	Moffatt, Greig, Valcanis, Gao, Seemann, Howden & Kirk, 2016
2012	WA	Pâté	4	Restaurant	OzFoodNet, 2012b
2012	SA	Chicken liver pâté	15	Restaurant	Parry, Fearnleyab & Denehya, 2012
2012	ACT	Chicken liver pâté	7	Private residence	OzFoodNet, 2012a
2011	WA	Duck liver pâté (baked to an internal core temperature of 60°C)	67	Restaurant	Merritt et al., 2011
2011	NSW	Chicken liver pâté on toast	11	Restaurant	OzFoodNet, 2015a
2010	SA	Steak with chicken liver pâté	18 (2)	Restaurant	OzFoodNet, 2011
2009	TAS	Chicken pâté (only pan fried with pink interior)	44	Restaurant	Merritt, Combs, & Pingault, 2011
2008	QLD	Chicken liver pâté	4	Restaurant	OzFoodNet, 2009
2007	QLD	Duck pâté	8	Restaurant	OzFoodNet, 2008

Poultry Liver Processing

Poultry liver is available at supermarkets, butchers and poultry retail outlets. Poultry liver is very cheap, usually only a couple of dollars per kilo and can be sold pre-packaged or unpackaged.

At the poultry abattoir, liver is removed by machine and then visually inspected. Damaged livers are removed manually, and the remaining livers are rinsed with chlorinated chilled water to remove any loose organic matter (a NSW poultry abattoir, personal communication, 2016).

Aim

The aim of this survey was to gather information on the prevalence and level of *Campylobacter* on the external surface and internal part of poultry livers sold in NSW. Other pathogens and microbiological indicator organisms were also tested. This survey was not conducted for enforcement purposes.

Materials and Method

Samples were purchased between March 2015 and December 2016. In total, fifty-one batches of poultry liver were purchased from supermarkets and butchers, comprising 50 batches of chicken liver and one batch of duck liver. For the purpose of this survey, a batch was either a pre-packaged container of livers (usually around 500g) or approximately 300g of liver purchased unpackaged. Samples were photographed and all sample information was recorded. Samples were sent under temperature control to DTS Food Assurance for testing within 24 hours of purchase.

Five individual livers from each batch were tested for their microbiological quality. Each liver was weighed and rinsed in 100ml of peptone saline. 1ml of this rinse was taken to test for *E. coli*. Another 0.1ml was used for *Campylobacter* enumeration and the remainder of the rinse was added to 400ml Bolton broth for *Campylobacter* presence/absence testing (reported as detected or not detected per 100ml).

The liver was then dipped in boiling water for 15 seconds to sterilise the outside surface. The liver was then diced and 10g was added to 90ml of peptone saline and stomached. Once stomached, 0.1ml was taken for *Campylobacter* enumeration and the remainder was added to 400ml Bolton broth for *Campylobacter* presence/absence testing (reported as detected or not detected per 10g).

One liver from each batch had its pH and water activity measured.

Results

A total of 255 livers from 51 batches were tested. The pH and water activity ranged from 5.21 to 6.44 and 0.97 to 0.99 with an average of 5.99 and 0.99, respectively.

The prevalence of *E. coli* was high with 58.4% (n=149) of livers having detectable levels of *E. coli* (>10 cfu/ml). Over 10% of individual livers (n=26) had counts of *E. coli* greater than 10^3 cfu/ml and two samples had counts up to 10^5 cfu/ml. Eleven batches had no detectable *E. coli.*

Individual liver results

The prevalence of *Campylobacter* in chicken livers was very high; A total of 96% of the individual liver was tested positive for *Campylobacter* (*Campylobacter* was detected both externally and internally in 88% of samples). Interestingly, two livers which returned 'not detected' results for the surface had enumeration results for the surface of 2,600 cfu/ml and 100 cfu/ml.

Table 2: Individual liver presence/absence results for *Campylobacter*

		Internal part of the liver		Total (for external surface)
		Detected	Not detected	
External surface	Detected	88.2% n = 225	3.9% n = 10	92.2% n = 235
	Not detected	4.3% n = 11	3.5% n = 9	7.8% n = 20
Total (for internal part of the liver)		92.5% n = 236	7.5% n = 19	n = 255

Only 28 (11%) individual livers from 15 batches had enumerable levels of *Campylobacter* externally and internally (enumeration sensitivity for *Campylobacter* was 100 cfu/g internally or 100 cfu/ml externally). In general, the level of *Campylobacter* was higher on the outside of the liver compared to the inside. Two samples had higher levels internally than externally (1600 cfu/g vs 300 cfu/ml, and 200 cfu/g vs 100 cfu/ml). These samples came from different batches.

Table 3: Individual liver quantitative results for *Campylobacter*

		Internal part of the liver		**Total (for external surface)**
		≥ 100 cfu/g	**< 100 cfu/g**	
External surface	**≥ 100 cfu/ml**	11.2% n = 28	48.0% n = 120	59.2% n = 148
	< 100 cfu/ml	3.6% n = 9	37.2% n = 93	40.8% n = 102
Total (for internal part of the liver)		14.4% n = 36	85.2% n = 213	n = 250*

*5 samples were not large enough to be tested quantitatively for both external and internal.

Batch results

All batches (n=51) tested had at least one liver with *Campylobacter* detected, meaning no batch was free from *Campylobacter.* Most batches (84.4%) had *Campylobacter* detected in all five livers, either on the external surface or internally, with 68.6% of batches having *Campylobacter* detected on both the external surface and the inside of all five livers.

Table 4. Presence of *Campylobacter* in a batch

Positives per batch		Number of individual liver in a batch positive for *Campylobacter* internally					
		5	**4**	**3**	**2**	**1**	**0**
Number of individual livers in a batch positive for *Campylobacter* externally	**5**	68.6% n =35	2% n = 1	2% n = 1	2% n = 1	-	-
	4	9.8% n = 5	7.8% n = 4	-	-	-	-
	3	-	2% n = 1	-	2% n = 1	-	-
	2	-	-	2% n = 1	-	-	-
	1	-	-	-	-	2% n = 1	-
	0	-	-	-	-	-	-

Although *Campylobacter* was detected in the majority of livers, they were mostly below the limit of quantification. Only one batch (2%) had five livers that had quantifiable levels both on the external surface and internally. Nine batches (17.6%) had no livers with quantitative levels externally or internally.

Quantifiable levels of *Campylobacter* were more likely to be obtained on the outside of the liver. For example, 78.4% of batches (n=40) had quantifiable *Campylobacter* for at least one liver externally compared to 41.2% of batches (n=21) that had quantifiable *Campylobacter* for at least one liver internally.

Table 5. Quantitative results of *Campylobacter* per batch

Positives per batch		**Number of individual liver in a batch with an internal count of *Campylobacter* ≥ 100 cfu/g**					
		5	**4**	**3**	**2**	**1**	**0**
Number of individual livers in a batch with an external count of *Campylobacter* ≥ 100 cfu/ml	**5**	2% n = 1	2% n = 1	2% n = 1	7.8% n = 4	9.8% n = 5	13.7% n = 7
	4	-	-	-	-	2% n = 1	5.9% n = 3
	3	-	-	-	2% n = 1	2% n = 1	11.8% n = 6
	2	-	-	-	3.9% n = 2	3.9% n = 2	5.9% n = 3
	1	-	-	-	-	-	3.9% n = 2
	0	-	-	-	-	3.9% n = 2	17.6% n = 9

Discussion

Campylobacter is found at varying prevalence in different foods. Its presence in livestock and meat, particularly poultry, is well documented (Cox et al., 2007). In chickens, *Campylobacter* colonises the mucous overlying the epithelial cells primarily in the caeca and the small intestine but may also be recovered from elsewhere in the gut and from the spleen and liver (The poultry site, 2013). Experimentally, the dose of viable *Campylobacter* required to colonise chicks can be as low as 40 cfu. Once colonisation is established, *Campylobacter* can rapidly reach extremely high numbers in the caecal contents to as high as 10^9 cfu in both experimentally challenged and naturally contaminated birds (The poultry site, 2013).

There have been various interventions at the different stages of poultry production to attempt to reduce *Campylobacter* contamination (Wideman et al., 2015). These interventions include an increase in on farm biosecurity, preslaughter management, improvements in the mechanics of slaughter and evisceration, carcase chilling and carcase chemical decontamination. However, these interventions have been directed towards flocks or poultry meat, and not poultry offal.

It appears that *Campylobacter* prevalence in poultry liver is quite varied from country to country. The prevalence of *Campylobacter* in poultry liver tested in this project was very high at 96% overall. This is similar to a New Zealand study (Whyte et al., 2006) which found 90% of livers tested had internalised *Campylobacter*. A Scottish study which only examined external prevalence found 81% of poultry livers purchased at retail were positive for *Campylobacter.* Molecular source attribution also demonstrated that strains from chicken liver were most similar to those found commonly in humans (Strachan et al., 2012). A Chilean study found 92.9% prevalence in frozen livers (Fernandez and Pison, 1995). A Belgian study found a prevalence of 61.7-74.6% (Ghafir, China, Dierick, Zutter & Daube, 2007). A Portugeuse study found a lower internalised prevalanced of 60% (Lemos, Morais, da Conceiçao Fontes, Pires and Vieira-Pinto, 2015). The difference between this survey and the current survey was that the Portuguese survey acquired livers direct from the abattoir and sterilised the surface by dipping in alcohol for 10 seconds, whereas this current survey acquired livers from the retail environment and sterilised the surface by immersing in boiling water for 10 seconds. A study in the USA found 48% of samples had detectable levels of *Campylobacter* on the surface only, 15% of samples had detectable levels of *Campylobacter* both on the surface and inside the livers, and only 1.7% of samples had detectable levels of *Campylobacter* inside the liver only (Barot et al., 1983). Mackiw, Rzewuska, Stos, Jarosz and Korsakl (2011) found a much lower prevalence of 31.4% on livers and 1.7% in ready to eat pâté in Poland.

The difference in prevalence may be due to differences in pre- and post-slaughter processes and conditions in the different countries. Baumgartner and Fellsien (2011) found that in Switzerland *Campylobacter* counts on livers increased from 10% in the cooler months to 100 % during the warmer months.

The livers tested in this survey were purchased at retail and while they are representative of what the consumer takes home, prevalence may be different compared to immediately post slaughter and post rinse. It would be useful to examine prevalence of *Campylobacter* immediately post slaughter and post rinse, both externally and internally, to determine whether there are interventions that can be made at the processor to reduce the level of *Campylobacter* in poultry livers e.g. increase chlorine level in rinse water or extend length of rinse.

There are limited studies on the level of *Campylobacter* detected on the surface and the inside of chicken livers. In this project, *Campylobacter* was detected at the level of greater than 10^3 cfu/ml in 12.3% of the surface of chicken livers tested. This is lower than a New Zealand study (Whyte et al., 2006) which found 30% of chicken liver surfaces sampled had greater than 1.1 x10^3 cfu/sample, but higher than a UK study (Firlieyanti et al., 2016) which found 2.8% of retail chicken liver surfaces had *Campylobacter* greater than 10^3 cfu/g.

As for the *Campylobacter* level inside the chicken liver, this project found that only 1.6% of samples had *Campylobacter* at the level of greater than 10^3 cfu/g. This is similar to the findings from the New Zealand and the UK studies which found 6% and 4.6% of samples had *Campylobacter* levels of greater than 10^3 cfu/g inside the chicken livers, respectively (Firlieyanti et al., 2016; Whyte et al., 2006).

Processing at consumer end to reduce *Campylobacter*

Liver as an organ can concentrate microorgansims and post-slaughter provide an ideal medium for microbial growth with high water activity and neutral pH. *Campylobacter* does not grow below 28°C and although its viability decreases during chilled storage, cells can still persist after several weeks of storage at chilled or frozen temperatures (Harrison et al, 2013). Chicken livers are often undercooked to retain some pink colour inside. Given the very high prevalence of *Campylobacter* in poultry liver, undercooking is a very hazardous practice. It is undercooking that primarily contributes to outbreaks linked to chicken liver pâté and parfait.

Whyte et al (2006), concluded that chicken livers need an internal temperature of 70°C for 2 to 3 minutes to kill *Campylobacter*. Harrison et al (2013) determined that freezing at -25°C for 24 hours can reduce numbers of *Campylobacter* by 2 logs. Reduction was greatly increased with an additional cycle of freezing (although quality of final product was not investigated).

Hutchison, Harrison, Richardson & Tchorzewska (2015) suggested a protocol for the commercial preparation of pâté which included freezing the livers and using a bain marie to cook to a critical temperature of 63°C. The bain marie heated the livers more uniformly. Sensory assessments in this experiment also determined that pâté made from frozen livers was preferred.

A number of foodborne outbreaks in Australia have been linked to poultry liver dishes, so Food Standards Australia New Zealand published a factsheet on how to cook poultry liver dishes safely (FSANZ, 2017). The factsheet states that whole livers need to be cooked to an internal temperature (measured using a digital probe thermometer) of 70°C for at least two minutes. They may still be slightly pink in the centre, but they should never be bloody or look raw. In addition, the safest way to prepare pâté is to follow recipes that require baking the whole dish in an oven or water bath, often at temperatures above 150°C for up to two hours. These methods should allow the livers to reach internal temperatures that would kill *Campylobacter.*

Conclusion

Poultry liver purchased in NSW retail stores has a high prevalence of *Campylobacter*. *Campylobacter* can also be internalised in poultry liver to illness causing levels making careful handling and adequate cooking of poultry liver critical food safety steps in the production of pâté and other products made from chicken liver.

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Appendix 1

International outbreaks attributed to poultry liver dishes

Year	Country	Vehicle	Cases (hospitalisation)	Setting	Reference
2014	USA	Chicken liver pâté	4	Restaurant	CDC, 2015; Scott et al., 2015
2012	Sweden	Chicken liver pâté	44	Wedding reception	Lahti, Lofdal, Agren, Hansson & Ogvall, 2016
2012	UK	Duck liver pâté	45	Catered wedding	Young et al., 2104
2012	USA	Chicken liver mousse	6(2)	Restaurant	CDC, 2013
2011	England	Chicken liver pâté	49	Wedding reception	Edwards et al., 2014
2011	UK	Duck liver pâté	32	Catering college restaurant	Abid et al., 2013
2010	England	Chicken liver parfait	24	Wedding reception	Inns et al., 2010
2009	England	Chicken liver pâté	59	Conference	Wensley & Coole, 2013
2006	Scotland	Chicken liver pâté	48	Restaurant	O'leary et al., 2009
2005	Scotland	Chicken liver pâté	86	Farming community	Forbes et al., 2009

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